DOES METABOLIC REPROGRAMMING UNDERPIN AGE-ASSOCIATED CHANGES IN T CELL PHENOTYPE AND FUNCTION?

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Key words- Redox; Treg; ageing; thioredoxin; protein oxidation; mitochondria
Abstract

T cells are required for an effective adaptive immune response. The principal function of T cells is to promote efficient removal of foreign material by identifying and mounting a specific response to non-self. A decline in T cell function in ageing is thought to contribute to reduced response to infection, vaccination and an increase in autoimmunity. This may in part be due to the age-related decrease in naïve CD4⁺ T cells and increase in antigen-experienced CD4⁺ T cells, loss of redox homeostasis and impaired metabolic switching. Switching between different subsets is triggered by the integration of extracellular signals sensed through surface receptors and the activation of discrete intracellular metabolic pathways.

The present article explores how metabolic programming and loss of redox homeostasis during ageing may contribute to age-associated changes in T cell phenotype and function.

Highlights

- Ageing T cells are hyporesponsive to specific stimulus and refractory to apoptosis.
- Anergic and regulatory T cells derive most of their energy from oxidative phosphorylation.
- The intracellular T cell redox environment affects exofacial protein expression and function.
- mTOR regulates the switch from oxidative phosphorylation to glycolysis, providing energy for expansion.
- Understanding the role of redox state in bioenergy shifts may support new approaches to improve specific immunity during ageing.

1. Introduction

The circulating T cell pool is highly diverse and derives from bone marrow stem cells which undergo maturation in the thymus (Figure 1; and discussed in section 2 of this review). T cell subsets can appear confusing; they are simply defined by the cytokines they secrete and the presence of specific cell differentiation (CD) antigens e.g. CD3 is expressed on all T cells in humans and so T cells are referred to as CD3⁻. T cells rely on reactive oxygen species (ROS) as regulatory molecules (discussed in section 3 of this review) and there are several examples where failure to produce superoxide anion radicals effectively have been implicated in autoimmune conditions (1). This review will focus on members of the CD4⁺ family whose frequency and distribution changes with ageing and which have been implicated in age-associated immune decline (section 4).

Naïve CD4⁺ T cells can differentiate into the highly proliferative effector T cell arm (including T helper (h)1, Th2 and Th17) which provides acquired immunity to pathogens and undergo metabolic activation and clonal expansion. On the other hand, those which reactive to self-antigens would normally become non-responsive (anergic) during development and a subset of specialised regulatory T-cells (Tregs) which modulate the immune system are metabolically quiescent (2). The frequency of Tregs is increased in aged mice and humans thereby restraining immune responses to pathogens (section 4).

An increasingly recognised pathway for modulation of signalling in a number of cells is mediated by reactive oxygen species (ROS) through local oxidation e.g. of protein tyrosine phosphatases, thereby ensuring phosphorylating signals remain active for longer. The sources and nature of ROS that are important in modulating signalling are likely to be hydroperoxides derived from superoxide anion radicals produced by NADPH oxidase enzymes (NOX) and mitochondria (3); mitochondrial ROS production is further enhanced when cytochrome oxidase efficiency is lower e.g. during ageing (section 5). This change is further compounded by metabolic changes in older adults which have been attributed to a redistribution of body fat to visceral deposition and insulin insensitivity, providing an increase in fatty acid metabolic substrate concentrations for oxidative phosphorylation (Figure 2). In turn, an enhanced catabolic metabolism from substrate flux through the electron transport chain leads to increased mitochondrial ROS. This in turn creates a more oxidising intracellular redox state and modulates transcription factor activity, gene
expression and protein trafficking to the cell surface (section 4). Exofacial thiols are recognised as important regulators of T cell proliferation and NOX activity which increases extracellular $\text{O}_2^-$ is an essential step for restoring immune homeostasis through Th suppression and enhanced Treg activity (4); this is discussed in section 5. Switching between different T cell subsets is triggered by the integration of extracellular signals sensed through surface receptors and the activation of discrete intracellular metabolic pathways and the evidence for this is discussed in section 6. An age-associated re-programming of cellular metabolism combined with loss of redox homeostasis during ageing may contribute to age-associated changes in T cell maturation and function (sections 7, 8 and 9).

2. T cells and their maturation

T cells derive from hematopoietic stem cell precursors that migrate from the bone marrow to the thymus (Figure 1). In the thymus, T cell precursors undertake different maturation steps that drive the commitment to the T cell lineage and the differentiation into the alternatives T cell lineages. The first development step involves the Notch signalling pathway and maturation processes involve the expression of different molecules at the surface of the cell and the rearrangement of the T cell receptor (TCR) genes. Initially c-Kit (stem cell growth factor receptor), CD44 (adhesion molecule) and CD25 (α-chain of IL-2R) are expressed on the T cell surface and the cells start proliferating. When the cells stop proliferating the TCR genes start rearranging. According to the TCR gene rearrangement, two T cell lineages can be produced: α:β and γ:δ.

γδ T cells represent 10% of the T cells subsets and are characterised by the expression of a γ:δ TCR at the surface. The major feature about γδ T cells is that these cells do not require the antigen to be presented in the MHC context and, consequently, do not need the presence of antigen presenting cells (APCs) to initiate an immune response.

The α:β T cells express a α:β TCR at the surface and, after positive selection, can differentiate into CD4 or CD8 T cells (5). CD4 T cells recognise pathogen antigens bound to major histocompatibility complex (MHC) class II (MHC-II) molecules and are described as T helper (TH) cells. CD8 T cells recognise pathogen antigens bound to MHC class I (MHC-I) molecules and are also known as cytotoxic T (Tc) cells (6).

During all the maturation process in the thymus, the T cells with TCRs that strongly interact with self MHC are negatively selected and eliminated. The cells that bear α:β TCRs that only recognise self MHC molecules are positively selected and leave the thymus to the blood stream as naïve T cells (5).

The contact between naive T cells and antigen presenting cells (APC) is highly specific. The TCR at the surface of T cells recognises a specific antigen bound to MHC molecules at the surface of the APCs (7, 8). However, the binding between TCR and MHC is not sufficient to activate the T cell and trigger an immune response. Costimulatory molecules and chemical mediators expressed by APCs are also required to activate T cells. Costimulatory molecules, such as CD28, CD80 and CD86, will engage with counter-receptors in the surface of T cells and transmit important signals for T cell proliferation and survival.

When the antigen presented by MHC binds to the TCR, intracellular protein tyrosine kinases are recruited, many of which are redox regulated (9). The activation of these signalling pathways results in the mobilisation of transcription factors that are crucial for gene expression and are essential for T cell growth and differentiation into effector T cells, ultimately leading to pathogen destruction (10-12).

3. A role for redox in T cell maturation

Ascorbic acid (vitamin C) is widely considered to enhance immune function, but evidence and mechanisms are lacking. However, a recent report has identified a role of ascorbic acid in functional T cell development. Ascorbate enhanced T-cell development independently of TCR rearrangement and genes encoding the co-receptor CD28 and its downstream kinase ZAP70 were upregulated by ascorbic acid. This may be occurring at the level of chromatin
demethylation, mediated by Jumonji C domain enzymes that require ascorbate as a cofactor rather than through an alteration of redox per se (13).

CD4+ T cells that become activated can develop into Th1, Th2, Th17 and T regulatory (Treg) cells, according to the dose of antigen presented by APCs, the type and level of costimulation and the cytokine environment that surrounds the CD4+ T cells (14). The Th1 response is particularly important against intracellular pathogens. Th1 cells secrete interferon (IFN)-γ, lymphotoxin α (LTα) and interleukin (IL)-2 and express CD40 ligand (CD40L) at the surface, leading to the activation and death of infected macrophages. The main function of Th2 cells is the induction of B cell proliferation and mediation of the immune response against extracellular parasites. Th2 cells produce B-cell growth factors, the interleukins IL-4 and IL-5 and CD40L (6, 15, 16). Th17 cells are involved in the immune response against extracellular bacteria and fungi and produce IL-17, IL-21 and IL-22. Th17 cells also contribute to the immune response observed in several autoimmune diseases, such as multiple sclerosis and rheumatoid arthritis (RA) (16, 17). The main function of Treg cells is to maintain the immune system tolerance to self-antigens. Treg cells secrete transforming growth factor (TGF)-β, IL-10 and IL-35, but they also induce immune responses through cell-cell interactions (16).

A recent study has suggested that NOX2 plays an important role in T cell maturation and defining CD4+ lineage; in NOX2-deficient mice, enhanced IFN-gamma and diminished IL-4 cytokine profile was observed with increased ratio of expression of a Th1-specific transcription factor rather than the Th2-specific transcription factor, consistent with a skewing of naive CD4+ T cells towards Th1 when extracellular ROS are low. In support of a role for the redox dependence of maturation, exposure to antioxidants inhibited, while pro-oxidants augmented Th2 cytokine secretion and STAT5 phosphorylation suggesting that TCR-induced NOX2-dependent ROS generation can control adaptive immunity (18).

In a previous study by King et al. peripheral blood mononuclear cells were stimulated with the ROS generator 2, 3-dimethoxy-1, 4-naphthoquinone with Th2 and Th1 phenotypes were promoted and inhibited respectively (19). Moreover, in the absence of APC, reactive carbonyls including 4-hydroxy-2-nonenal and malondialdehyde, which are generated on proteins and lipids in the presence of ROS, promote differentiation towards a Th2 phenotype (20). These data emphasise the importance of ROS homeostasis and flux in governing cell maturation, and that the balance between oxidising and reducing agents is a delicate process which must be tightly regulated and well managed, depending on whether the requirement is for protecting against bacteria, in an immune response, or requirements for T-cell signaling, activation and regulation of function.

The redox environment at the interface between APC and T-cells within the immunological synapse also impacts on T-cell activation. Activated T-cells exhibit increased cell surface thiol levels suggesting that a reduced extracellular environment is required for T-cell activation (21). Moreover, DC have been shown to create this reducing environment by releasing cysteine into the extracellular space thereby facilitating an immune response (22, 23). Tregs may exert their immunosuppressive effect by interfering with this process by rapid uptake of available extracellular cystine for glutathione (GSH) synthesis (24-26). Another study showed that T-cell proliferation in response to antigenic stimulation in selenoprotein deficient T-cells isolated from mice is suppressed (27). Conversely, CD4+ T-cells isolated from mice fed either a diet of high, medium or low selenium for eight weeks exhibited increased proliferation and expression of IL-2 and IL-2 receptor in response to antigenic stimulation; CD4+ T-cells from low-selenium diet mice had lower proliferative response, lower intracellular thiol and iGSH and response to antigenic stimulation was rescued by N-acetyl cysteine (28). Thus altered intracellular redox state, as well as levels at the interface between APC and T-cells at the cell surface, can impact on T-cell activation, proliferation and differentiation. This has wider implications for ageing and disease which are associated with oxidative stress.

TCR engagement with the specific antigen presented APCs activates naïve T cells and they differentiate into effector T cells. Effector T cells will then induce B cells to expand and differentiate into antibody producing cells (7, 29). The antibodies produced by these cells will recognise specifically the antigen that triggered their production and ultimately will lead to the antigen destruction (7, 29). Most activated T and B cells die once the immune response is
complete (6). However, some of these cells differentiate into memory cells and after a second exposure to the antigen they trigger a faster and stronger secondary immune response (7). Consistent with the requirement to respond rapidly to reactivation it has been suggested that such memory cells require a rapid energy supply and anti-TCR antibody activation of memory T cells has been shown to increase their chemotaxis to CCL5, which was dependent predominantly on glycolysis rather than fatty acid oxidation (30). Nutrient supply and regulation plays an important role in T cell activation.

4. The ageing immune system

Several physiological systems including cells of the immune system lose their homeostatic capacity with age (31, 32). Typical dysfunction in immune responses relate cellular dysregulation, such as impaired phagocytosis by neutrophils (33) and in T cells, reduced levels of TCR/CD28 receptor expression due to transcriptional inactivation (34), a decrease in the Th1:Th2 ratio (35) and skewing of immune effector pathways by persistent pathogens such as cytomegalovirus that stimulate futile clonal expansion and senescence (36). Consequently, ageing T cells are considered to be hyporesponsive to stimulus and refractory to apoptosis, a phenomenon that we have previously associated with altered redox state (1). This is compounded by accumulating naturally occurring Tregs with advancing age, but loss of inducible Tregs (37). Exceptionally long-lived animals appear not to show typical features of immune senescence, have controlled levels of oxidants and regulated nuclear factor-κB (NFkB) activation implicating these pathways in immune ageing (38). Indeed, loss of the antigen presenting co-receptor CD28 on T cells can be accelerated by prolonged exposure to the proinflammatory cytokine tumour necrosis factor (TNF)-α and normal chronological ageing is associated with low level, but persistent, elevation of TNF-α (39).

T cells work in concert with several other immune cells including antigen presenting cells, e.g. dendritic cells (DC) and B cells. DCs travel to peripheral lymphoid organs and interact with T-cells creating an immunological synapse and providing the necessary signals for T-cell activation: First the T-cell receptor (TCR) engages with antigen-bearing MHC molecules; second, the co-stimulatory molecules, especially CD80 and CD86, bind co-receptor CD28 on surface of T-cells; and third cytokines are produced to orchestrate the adaptive immune response. While the focus of this review will be on T cells, it is also pertinent to note that both reducing and oxidising molecules are released into extracellular environment by other cells relevant to immune ageing, including DCs, macrophages and neutrophils (4).

5. Ageing, ROS and redox

Many theories have been explored in animal models to explain the increase in frailty with age. At the molecular and cellular level; accumulation of damaged molecules, loss of cell replicative capacity or senescence, repair deficits and impaired nutrient response pathways have been considered as key drivers of ageing (40). No single theory has proved sufficient to explain human ageing probably because the pathways are largely interdependent. For example, molecular damage accumulates when prevention and/or repair and/or autophagy are ineffective. Genotoxic damage arising from oxidative stress was originally proposed by Harman in the 1950s as a cause of ageing and is also an inducer of the senescent phenotype, which associates with decline in metabolic homeostasis and immune function. More recent studies in model animals including the nematode worm and drosophila have consistently have identified the daf-2 homologue, the insulin growth factor, working with the daf-16 homologue, FOXO, as a critical component of the ageing process that together control metabolic pathways, regulate response to stress and resistance to bacterial pathogens (41).

Not all oxidation reactions contribute to damage accumulation and many are critical for physiological signalling. Such oxidation tends to be reversible and can be restored through action of specific redox couples. Each redox couple can act as a unique rheostat on/off switch, regulating specific cellular protein activity through oxidation and reduction (42); Figure 3. Indeed distinctive redox nodes exist in specific subcellular concentrations and compartments. Thus, disruption of redox state in one compartment may uniquely affect the activity of local cell signalling (43).
Oxidised and reduced thioredoxins (Trx) are important redox couples which are central to a network of redox couples including glutaredoxin, sulphiredoxin and peroxiredoxins. Trx is restored from its oxidised form by Trx reductase in an NADPH-dependent manner. Interestingly, loss of the mitochondrial isoform Trx2 does not affect lifespan (44). However, Trx1 knockout is embryonic lethal (45) and the median lifespan of mice over-expressing human cytosolic Trx1 is greater than for wild-type mice (46, 47).

Trx1 is a small, 12-kDa, conserved and ubiquitous multifunctional protein with several redox-active cysteine residues. It acts as an antioxidant, anti-inflammatory agent and redox-regulating enzyme (reduces disulphide bonds and sulphenic acids but also uniquely to Trx1, has transnitrosylation activity) (48-51). Trx1 regulates chemokine activity, reduces inflammation, cellular infiltration, and LPS-induced oxidative damage (52-54). Furthermore, the longevity of Trx1 overexpressing mice is associated with a lower incidence of acidophilic macrophage pneumonia suggesting that Trx1 is an important anti-inflammatory and immune modulator (47). We recently reported that healthy older adults have reduced T cell surface expression of Trx1 which could be mimicked by depleting the intracellular reducing molecule glutathione (GSH) (55). Whether loss of T cell surface Trx1 has any effect on surface thiols in older adults is not known.

Trx1 has many interaction partners depending on its cellular localisation. The most energetically and physiologically favourable reaction for Trx1 is to reduce oxidised peroxiredoxins within the redox network (56). Through its reductase activity it may regulate apoptosis, cell growth, differentiation, migration, angiogenesis, tumorigenesis, and development (45, 57). In the nucleus, Trx1 binds directly to different transcription factors and thereby modulates their DNA-binding activity, e.g., p53, NFkB, and AP1 (58, 59). With respect to apoptosis inhibition, at least three binding partners have been identified in the cytoplasm; the apoptosis signaling kinase 1 (ASK-1), the Trx-interacting protein (TXNIP) and actin, where actin protects Trx1 from degradation and preserves its anti-apoptotic function (57, 60). Trx1 also associates with the plasma membrane; it is trafficked with a limited number of cytosolic proteins via the leaderless secretory pathway, with anchorage in the membrane probably mediated by palmitoylation of cysteine (61). Trx1 may also be secreted, exerting a range of effects on T cells, B cells and fibroblasts from growth arrest to autocrine activation of T cells (62); extracellular Trx1 influences the redox state and function of ligands such as IL-4 (63) and maybe taken up by adjacent cells via lipid rafts when cysteine is oxidised (64).

During ageing, there is a progressive decline the ratio of cysteine to cystine and reduced to oxidised GSH in the plasma which has been attributed to excessive oxidants within a proinflammatory environment (65). Recent studies have implicated a cooperative interaction between the intracellular T cell redox environment and exofacial membrane proteins, which ultimately influences T cell function in health and disease (66, 67). A loss of redox homeostasis during ageing may contribute to age-associated changes in T cell phenotype, through altered gene expression, receptor oxidation state and therefore to aberrant function.

6. T cell activation; an interplay between metabolic and redox state

Anergic and Treg cells derive most of their energy from oxidative phosphorylation in a survival response process driven by IL-7. This catabolic metabolic pathway supports low energy requirements for housekeeping functions. Memory T cells mount a faster and stronger response to reinfection than naïve T cells do following initial infection probably through ATP production by a combination of glycolysis and oxidative phosphorylation as they have greater mitochondrial mass, the site of glucokinase and the respiratory chain, than naïve cells. This bioenergetic advantage supports rapid recall in response to reinfection (68). When presented with costimulatory factors, IL-7 can also drive T cell proliferation via glycolysis. This is achieved by regulating expression of hexokinase II (69) and through the PI3K-dependent upregulation of the glucose transporter GLUT1 (70). These findings support the wider importance of a metabolic control node for T cell differentiation and activation via a Warburg-like effect, a switch from oxidative phosphorylation to glycolysis even when oxygen is abundant (Figure 2).
Th1, Th2, and Th17 cells express high surface levels of the glucose transporter GLUT1 and are highly glycolytic. As CD4⁺ T cell subsets operate distinct metabolic pathways, they may be manipulated in vivo to control Treg and effector T cell development in inflammatory diseases (71). Leptin may moderate susceptibility to autoimmune disease through its effects on the survival of Th1/Th17 cells and the inhibition of proliferation of Treg cells.

The key metabolic pathways of glycolysis and oxidative phosphorylation are intimately related to redox biology. Glycolysis is the principal source of NADPH and is linked to control of the NADH/NAD⁺ redox couple. NADPH is an essential cofactor for the NADPH oxidase on the one hand and for glutathione and Trx regeneration via their respective reductases. NAD⁺ is an essential cofactor for poly-ADP ribosyl transferase and for sirtuins which exert epigenetic control over gene expression through regulating acetylation of transcription factors (Figure 4).

Cellular redox balance is achieved via three major redox couples; NAD-NADH; NADP-NADPH and the cysteine containing tripeptide, GSH - oxidised glutathione (GSSG) (72). Cellular GSH concentration is dependent on the activity of gamma-glutamyl cysteine ligase (GCL) and cysteine availability (73); expression of the rate limiting GCL enzyme is coupled to cellular redox state through the Nrf-2-KEAP1 system, providing a mechanism for cellular adaptation to oxidative stress through de novo GSH biosynthesis (73). Therefore a decrease in protein thiols e.g. through oxidation should give rise to an increase in de novo GSH synthesis so that cellular redox state is restored.

An increase in intracellular oxidised GSSG can normally be minimised by promoting its efflux via multidrug resistance-associated proteins (74). In addition, organelles such as the mitochondrion (which also express Trx 2 uniquely) and the nucleus maintain active transport processes for GSH to preserve a local reducing environment against concentration gradients as required for cell proliferation, active gene transcription and to minimise damage from ROS leakage during respiration (43, 74). The efficiency of Trx1 is likely to be of particular significance during chronic inflammation when ROS/RNS production by phagocytes will favour a more oxidising extracellular environment (75).

Oxidative phosphorylation at the mitochondrion is the principal source of ROS in non-phagocytic cells, which is increased in ageing T cells due to loss of respiratory chain integrity at cytochrome oxidase. Retrograde signaling from the mitochondrion to the nucleus or cytosol controls cell growth and differentiation. Indeed, mitochondrial metabolism in the absence of glucose metabolism is sufficient to support IL-2 induction required for proliferation, probably via epigenetic control. Using mice with reduced mitochondrial ROS (mROS) production in T cells, Sena et al showed that mitochondria were essential for T cell activation via mROS-dependent activation of nuclear factor of activated T cells (NFAT) and subsequent IL-2 induction. T cells retained the ability to proliferate in vivo and were not lacking bioenergetically but instead were deficient in specific ROS-dependent signalling events needed for antigen-specific expansion. Together these data suggest that controlled mitochondrial metabolism is a critical component of T cell activation through the production of ROS from complex III (76).

Signals such as IL-2 will act on T cells promoting their differentiation into effector cells in a redox-sensitive manner (8). In contrast to primed cells, PKC-theta, a high-molecular disulfide-linked complex found at the plasma membrane in naive T cells, plays a central role in TCR-induced IL-2 production and T-cell proliferation. During T cell activation, with glutathione and Trx, PKC-theta is reduced to the active monomeric form (77).

We have previously reported that loss in intracellular GSH during hypoxia enhanced T cell interleukin 2 receptor expression in response to phytohaemagglutinin (78). This was most likely from mitochondrially derived ROS and is consistent with previous observation (79). In contrast, using plumbagin, a thiol depleting agent that increases cytosolic ROS, mitogen-induced T-cell proliferation and cytokines (interleukin (IL)-2/IL-4/IL-6/interferon-gamma) production was suppressed and this effect was reversed by thiol antioxidants but not by non-thiol antioxidants (80). Buthionine sulfoximine (BSO), an inhibitor of GSH synthesis, also markedly reduced T cell proliferation without affecting viability and blocked production of IL-2 and IL-6 (21, 55). In contrast, others have shown that BSO could not inhibit IL-2 production i.e. lymphocyte activation but did inhibit cell cycle entry and proliferation (81, 82). There is a complex picture emerging around thiols, ROS and T cells that may be explained in part by redox state in specific
compartments and activation stimuli received by cells; exogenous glutathione has been shown to inhibit IL-2 synthesis in mitogenically stimulated T cells although was required for DNA synthesis by Roth and Droge (83), but in contrast exogenous glutathione decreases IL-4 but not IL-2 production in peripheral blood lymphocytes (84).

We recently reported that healthy older adults have reduced T cell surface expression of Trx1 and lower circulating plasma Trx1 concentrations (55). Together these data suggest that a relationship exists between the intracellular redox compartment and extracellular Trx1. Loss of lymphocyte surface Trx1 may lead to increased oxidation of membrane proteins and receptors, in turn influencing cellular responses to activating stimuli (85). In a proteomic study of the effect of ascorbic acid on CD4+ T cells, we showed that expression of proteins were associated with signalling, carbohydrate metabolism, apoptosis, transcription and immune function was altered and that signalling was also influenced physiologically following dietary intake of vitamin C in healthy adults (86, 87) providing further evidence for links between metabolic and immune phenotype.

7. Insulin signalling, mTOR and ageing T cells

Many mutations that extend life span in worms, flies, and mammals perturb endocrine signalling. The best understood of these signaling pathways is the insulin/IGF-1 pathway which extends lifespan when knocked-down (88). Ironically, in older adults, insulin signalling is already impaired and can precede the development of diabetes. This could be an adaptation to slow cellular ageing, although the consequences of elevated glucose concentrations in extracellular compartment are also negative and include increased ROS production through interaction of glycated proteins with receptor for advanced glycation end products (RAGE). The consensus view is that maintaining insulin sensitivity is better for health although this may accelerate cellular ageing.

CD4+ T cells express insulin receptor and IGF-1R, and after cell activation receptor expression is downregulated and inversely correlated with CD25, an IL2R, marker of activation and highly expressed on Treg (89). In turn, IGF-1R is linked via phosphoinositide 3-kinase (PI3K) to the mechanistic target of rapamycin (mTOR), an evolutionally conserved serine and threonine kinase, which plays a critical role in the promotion of cell growth and proliferation. In adaptive immunity, the mTOR pathways (mediated via two related protein complexes TORC1 and TORC2) coordinate the metabolic regulation of naïve T cells in non-proliferative state, to their subsequent activation and differentiation following antigen recognition. mTOR acts as an environmental sensor, linking immune signaling with nutrient availability (e.g. cysteine, glutamine, tryptophan) and activates aerobic glycolysis so enabling the cell to switch to rapid energy production in support of proliferation (90, 91). Interestingly rapamycin itself, which is considered an anti-ageing molecule, was first considered as an immunosuppressive because of its antiproliferative effects. Some recent studies have elucidated the physiological role of metabolic reprogramming during T-cell activation and trafficking, which are potentially relevant to inflammatory disorders and cancer, partly under the regulation of NFkB and this has been precisely detailed in a recent review (92).

IL-2, IL-7 and CD28 receptor engagement activate the PI3K/Akt/ mTOR pathway to support survival, proliferation and growth of T cells. Proliferation is ROS dependent and relies on GLUT1-dependent glucose uptake for glycolytic production of NADPH. Both NADPH oxidase and mitochondrial respiratory chain-generated ROS contribute to proliferation (93).

Middle-aged obese and non-obese older adults share in common an increase in deposition of fat around visceral organs which contributes to insulin resistance and an increase in circulating insulin. As insulin signalling is important for T cell differentiation and can therefore influence local and distant immune responses, Winer et al examined the profile of T cells in visceral adipose tissue from mice following diet induced obesity. T cell receptor V(alpha) repertoires were biased, suggesting antigen-specific expansion in favour of Th1 cells. In contrast, immunotherapeutic transfer of CD4+ T cells reversed the diet-induced weight gain and insulin resistance, predominantly through Th2 cells. These findings suggest that the progression of obesity-associated metabolic abnormalities is at least in part under the pathophysiological control of CD4+ T cells (94).
In another study, high circulating levels of insulin, commonly seen in ageing and obesity, activate AKT signaling which inhibits both IL-10 production and the ability of Tregs to suppress TNF-α production by macrophages. While there was no effect on surface CTLA-4 or CD39 expression, these findings do suggest that high insulin impairs the ability of Tregs to suppress inflammatory responses (95). Eller et al also showed that obese patients with insulin resistance displayed significantly decreased natural Tregs but an increase in adaptive Tregs in their visceral adipose tissue as compared with lean control subjects (96). They further investigated whether there was any role of Tregs in control of glucose homeostasis using an anti-CD25 monoclonal antibody and observed that Treg-depleted db/db mice showed increased signs of diabetic nephropathy in addition to the previously described proinflammatory cytokine profile. Furthermore adoptive transfer of Tregs significantly improved insulin sensitivity and reduced diabetic nephropathy suggesting a potential therapeutic value of Tregs (96). Another obesity-associated hormone, leptin, may moderate susceptibility to autoimmune disease which is prevalent in older adults, through promoting survival of Th1/Th17 cells and inhibiting proliferation of Treg cells (97).

Considering these findings, it does not appear that insulin sensitivity alone can be responsible for determining cell differentiation pathway as T cell bias towards low energy demand would be expected in insulin resistant and diabetic subjects. Indeed, activating a T cell with insulin does not have the same functional consequences as CD28 activation of mTOR. Furthermore, recent observations indicate that effector T cells can polarise to a regulatory phenotype through stabilisation of Foxp3 by its acetylation; this is consistent with increased availability of acetyl CoA and decreased SIRT1 activity during nutrient excess (98, 99). Conversely, a recent study described how resveratrol, a SIRT activator, decreased CD4+ activation and correspondingly the severity of arthritis in mouse model (100). Another important molecule, visfatin, secreted by visceral adipose tissue and increased in obesity acts as a phosphoribosyltransferase (a key step in the de novo production of NAD), regulates insulin receptor expression and has both metabolic (101) and immunoregulatory effects; intracellular NAD levels are important in regulating human T lymphocyte survival, IL-2 secretion, and the proliferative response to antigenic stimuli (102). Finally, in CD8 cells, a memory phenotype was induced by an ability to switch on fatty acid oxidation and was enhanced by metformin in AMPK deficient cells (103, 104). It will be of interested to explore whether T cell SIRT activity changes with age and to understand what is the effect of manipulating SIRT activity for T cell polarisation.

Further control over pathways of differentiation has been identified through the tryptophan metabolite kynurenine, which can be formed enzymatically by indoleamine 2,3-dioxygenase or from free radical oxidation of tryptophan. Through binding to the aryl hydrocarbon receptor, kynurenine interacts with HIF-1 and directs Th17 differentiation (105). Our previous work has shown that kynurenine is increased in circulating IgG from rheumatoid patients lending support to the presence of increased substrate availability, possibly generated via oxidative stress, for AhR to drive T cell differentiation down an autoimmune Th17 path (106). The key theme to emerge from these studies is that metabolites in general and nutrients specifically via mTOR provide a direct link between T cell metabolism and function. Indeed, mTOR also regulates autophagy as an alternate energy supply when nutrients are low (107). mTOR orchestrates the immune microenvironment and programmes the generation of CD4(+) effector versus regulatory/memory T cells shifting the metabolic profile of rapidly dividing and clonally expanding cells from fatty acid oxidation to glycolysis to meet the energy demand, so facilitating T cell trafficking, and T cell activation versus the low energy requirements of anergic cells (Figure 5). The reader is referred to a recent article where this has been reviewed very eloquently by Powell (108). Beyond this, a number of ‘metabolic checkpoints’ sense metabolic status and transduce signals to affect T lymphocyte responses as recently reviewed by Green (109). This suggests a potential to manipulate (auto) immune responses through metabolic intervention and so to reduce the age-associated immune dysfunction that results in increased auto-reactivity and decreased response to specific antigen.
8. T cell trafficking and the influence of metabolic and redox states

Once T cells have matured in the thymus, they are trafficked to the periphery in an energy requiring process. Their presence at inflammatory sites is a combination of their ability to respond to chemotactic recruiting stimuli, and to survive and proliferate locally. In order to explore the metabolic requirements for trafficking in response to chemotactic stimuli such as CCL19 and sphingosine 1 phosphate (S1P), Taub et al showed that inhibition of fatty acid oxidation but not basal glycolysis, significantly suppressed CCL19- and S1P-mediated adherence to collagen by >50 and 20%, respectively, and chemotaxis by >20 and 50% (30).

In considering redox involvement in migration, cofilin is one of the major regulators of actin dynamics in T cells and can be inactivated by oxidation leading to a hypo-responsive, non-migratory phenotype. In contrast, in a reducing environment, cofilin becomes active, drives dynamic changes in actin at the plasma membrane and promotes trafficking (110). This again highlights this significance of temporal regulation of specific redox compartments in cells to facilitate T cell function.

Treg accumulation at specific sites may result from aberrant proliferation and trafficking as well as greater resilience to oxidative stress compared with conventional T cells. This enhanced antioxidative capacity of Tregs possibly serves as feedback inhibition during inflammation and prevents uncontrolled immune reactions by favouring survival of suppressor rather than effector cells. Mougiakakos et al have demonstrated that human Tregs express and secrete higher levels of Trx1 (25). Similarly, in rheumatoid arthritis (RA), Szabo-Taylor et al have shown that Th17 cells expressed increased exofacial redox-regulating enzymes such as peroxiredoxin 2 which may be involved in the persistence of pro-inflammatory cells in chronic inflammatory sites by maintaining key receptors such as IL-2R in a reduced and biologically active state (111, 112). On the other hand, a recent study of RA T cells, including naive cells, showed them to be metabolically reprogrammed with insufficient up-regulation of the glycolytic activator phosphofructokinase FB3, so therefore energy-deprived, ROS- and autophagy-deficient, apoptosis-sensitive, and prone to undergo senescence (91). Prolonged exposure to high ROS concentrations can inhibit T-cell proliferation (113) and in the presence of uncontrolled ROS or inadequate nutrient supply at an inflammatory site, an ineffective response to pathogen is likely because of failure to mount an effective immune response.

9. Metabolic and redox homeostasis as a target for rejuvenating T cells and preventing age-associated disease

CD4+ T cells are critical drivers of age-associated pathogenic immunity. Understanding how environmental cues interplay with T cell metabolism and redox state to influence T cell fates will provide insight into the mechanism of immune responses, how ageing compromises immune function and for the development of novel therapeutics to target age-related immune decline.

Acknowledgments

The authors acknowledge financial support from the European Union through the Markage project “MARK-AGE (EU FP7 Large-scale integrating Project HEALTH-F4-2008-200880)”. RBDCT is funded by the European Union through the RAPID ITN consortium project. HRG is also acknowledges support through COST CM1001 and COST BM1023 programmes.

Abbreviations

APC, antigen presentation
BSO, buthionine sulfoximine
CD cell differentiation
GLUT, glucose transporter
GSH, glutathione
GSSG, oxidised glutathione
IFN, interferon
IGF-1R, insulin growth factor receptor 1
IL, interleukin
mROS, mitochondrial ROS
MHC, major histocompatibility complex
mTOR, mechanistic target of rapamycin
NFkB, nuclear factor kappa B
NOX, NADPH oxidases
RA, rheumatoid arthritis
ROS, reactive oxygen species
S1P, sphingosine 1 phosphate
TCR, T cell receptor
TGF, transforming growth factor
Th, T helper cell
Treg, Regulatory T cell
Trx, thioredoxin
TNF, Tumour necrosis factor

Legends

Figure 1. Pathways of T cell maturation and regulatory cytokine involvement in terminal differentiation.
Figure 2. Metabolic control nodes of glycolysis and oxidative phosphorylation and the Warburg effect.
Figure 3. Intra- and extra-cellular redox switches
Figure 4. Metabolism, NAD+ and epigenetic control in ageing.
Figure 5. The metabolic profile of rapidly dividing effector versus regulatory/memory T cells
References


PMID: 18544525. Epub 2008/06/12. eng.


Figure 1.

**Bone marrow**
- Lymphoid progenitor
- Stem cell

**Thymus**
- T cell precursor
- DN
- CD4
- CD8
- γδ

**Periphery**
- T helper
- T cytotoxic
- APC
- Th effector
- Tc effector

**Cell-mediated immunity and inflammation**
- Protection against intracellular pathogens (viruses, bacteria)
- Autoimmunity
- Inflammation

**Antibody-mediated immunity**
- Protection against extracellular parasites
- Asthma, allergy

**Protection against extracellular bacteria (skin, lining of intestine), fungi**
- Autoimmunity

**Immune tolerance**
- Lymphocyte homeostasis
- Regulation of immune responses

**Cytokines**
- IL-12
- IL-4
- IL-21
- IL-23
- TGFβ
- IL-6
- IL-17A
- IL-17F
- IL-21
- IL-22
- TNFα
- IFNγ
- IL-10
- IL-35
3. The Warburg effect describes how energy is produced by a high rate of glycolysis and is preferred by Th1, Th2 and Th17 cells.

1. T cell receptor activation, clonal expansion with high energy requirement

2. In ageing, intracellular NAD⁺ is increased SIRT1 activity is decreased and transcription factor acetylation is greater.

Fatty acid (FA) transporter e.g. CD36
Figure 3

[Diagram showing the structure of cysteine and its interactions with different processes such as catalytic nucleophile, redox catalytic, and regulatory e.g., Protein Tyrosine Phosphatase (PTP)].
Younger T cells
- Redox homeostasis maintained
- Surface Trx maintained
- More resistant to Treg inhibition by $O_2^-$, $H_2O_2$

Older T cells
- Greater mitochondrial uncoupling
- Lower intracellular and surface Trx
- Greater chance for surface thiol oxidation
- Impaired response to extracellular stimulus
- Depletion of intracellular NAD+
- Decreased SIRT1 activity
- Increased transcription factor acetylation
- Epigenetic modulation

Figure 4
Figure 5

Younger

APC
Peptide-MHC   B7

TLR   CD28

T cell

↑↑PI3K

↑AKT

↑↑mTOR

↑GLYCOLYSIS

Anabolic metabolism
↑NADPH  ↑GSH
↑CD4⁺ TH1 TH2 TH17
↑Proliferation
↑Migration to tissue

Older

APC
Peptide-MHC   B7

TLR   CD28

T cell

↑↑PI3K

Weak AKT Weak

↑OX PHOSPH↑

Catabolic metabolism
↑Mitochondrial O₂⁻
↑anergic Treg
↓Proliferation
↑Homing

Metformin

mTOR

Autophagy